Nanowell surface enhanced Raman scattering arrays fabricated by soft-lithography for label-free biomolecular detections in integrated microfluidics

Gang L. Liu and Luke P. Lee

Biomolecular Nanotechnology Center, Berkeley Sensor and Actuator Center, Department of Bioengineering, University of California, Berkeley, California 94720 and UCSF/UCB Bioengineering Graduate Group

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We describe a low-cost, ultrasensitive surface-enhanced Raman scattering (SERS) substrate in microfluidic biochips fabricated by soft lithography. A batch nanofabrication method is developed to create nanopillars structures on a silicon wafer as a master copy of molding, then the complementary nanowell structures on polydimethylsiloxane (PDMS) are created by soft lithography. The selective deposition of Ag thin film on the nanowells is applied to create SERS active sites before the integration with a glass-based microfluidic chip which functions as a sample delivery device and a transparent optical window for SERS spectroscopic imaging. Detections of Rhodamine 6G and adenosine SERS spectra are accomplished by using a 785 nm laser with 300 μW excitation power. The Raman scattering signal enhancement on the nanowell-based Ag SERS substrate is more than 10^7 times higher than the control sample (i.e., the smooth Ag layer on PDMS). Fabrication of ultrasensitive nanowell SERS substrate by economical and repeatable soft lithography method can contribute to the future microdevices for high throughput screening of functional genomics, proteomics, and cellular activities. © 2005 American Institute of Physics.

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Raman spectroscopy is an ideal label-free optical detection technique for chemical and biomolecules. The Raman spectrum is considered as the optical fingerprint of chemicals and biomolecules as it represents the vibrational frequencies of functional chemical bonds in molecules, and it has the advantages over infrared absorption spectroscopy since it has a capability to detect both symmetric and non-symmetric chemical bonds with negligible light absorption by water in visible and near infrared light region. While the extremely low efficiency of Raman scattering makes the detection difficult, surface enhanced Raman scattering (SERS) provides a solution to increase its optical cross section as close to fluorescence spectroscopy with optimized SERS substrates. Most current SERS substrates are colloidal Ag or Au nanoparticle clusters and they are widely used in the bulk-volume solution based chemical and biomolecule detections and recently in microfluidics. Nanoparticles assembly monolayer on silicon and polymer as well as E-beam fabricated nanoparticles array are used as SERS substrates on chip; however, the sample preparation of such substrates requires significantly serial, manual, and expensive efforts which thus hinder their applications on large-scale integrated microfluidic biochips. Recently, De Jesus et al. deposited Ag thin-film on smooth polydimethylsiloxane (PDMS) as a SERS substrate and demonstrated sub-nM concentration level detection sensitivity; however, the surface enhancement effect is modest in comparison with nanoparticle “hot spots” to create sensitive SERS substrates.

This letter provides solutions to create low-cost and ultrasensitive label-free SERS sensors array in integrated microfluidics by PDMS-based soft-lithography which allows a robust replication of optimized nanostructure molds for identical SERS substrates. In comparison with the on-chip SERS substrates made by E-beam lithography, our soft lithography-based nanofabricated SERS substrate is batch-processed and mass-reproducible since it can be repeatedly replicated from only one master copy. By knowing the optimization conditions of SERS substrates, first we fabricate nanostructures on silicon or glass substrate using conventional lithography and etching methods. Then, we choose the best master copy, and apply repeatable PDMS-based soft lithography and a simple metal deposition on the replicated nanostructures, which allows economical mass production of identical SERS active sites on polymer substrates. The background noise of Raman signals from the polymer substrate is avoided since the deposition of metal thin film (i.e., Ag or Au) for the formation of nanowell SERS structures blocks excitation light sources to pick up extra Raman signals from PDMS polymer substrate. Also a simple shadow masking process for selective thin film metal deposition on nanofabricated PDMS substrate provides an effective integration solution to bond with a glass-based microfluidic channel array chip. Raman spectra of Rhodamine 6G (R6G) and adenosine molecules down to sub-picomolar concentration level in microfluidic channels are detected on the nanowell replica-based SERS substrates. The Raman scattering intensity of the biomolecules on the Ag/PDMS nanowell structure shows the enhancement of more than 10^7 times in comparison to the smooth Ag/PDMS surface.

The pattern of SERS substrate area was designed to match that of the microfluidic network to be integrated with, and then created on a 4 inch silicon wafer with a 1-μm-thick polysilicon top layer by standard photolithography. In this particular case, we fabricated silicon nanopillar structures on the patterned area via a completely batch process reported previously, and we used them as the mold for the subsequent PDMS replications. Although the dimensions of the nanopillars are not perfectly uniform, the average density and aspect ratio of nanopillars can be controlled by plasma
etching processing parameters.\textsuperscript{14} We selected the silicon nanopillar with average pillar diameter of 40 nm and average pillar spacing of 44 nm which is close to the optimal SERS condition proposed by Garcia-Vidal and Pendry.\textsuperscript{15} The nanostructured mold used in this soft lithography-based SERS substrate fabrication is not limited to the silicon nanopillars. Any nanofabricated mold such as E-beam fabricated nanoparticles, nanoporous silicon, and self-assembly nanoparticles can be used as long as the optimal SERS conditions are implemented in the master copy of molding.

In order to keep the integrity of the nanoscale structure on PDMS during peeling off process, the silicon nanopillar was firstly salinized with an anti-stiction agent (tridecafluoro-1,1,2,2-tetrahydrooctyl)-1-trichlorosilane ($\text{C}_8\text{F}_{13}\text{CH}_2\text{CH}_2\text{SiCl}_3$) in a chemical vapor deposition chamber before casting PDMS. The PDMS layer was cured in room temperature and peeled off after complete solidification. The PDMS chip was treated with oxygen plasma for surface cleaning and bonding with glass chips. In the following, a 20 nm thin film of silver was selectively deposited on the nano-well PDMS surface using an electron beam evaporating, a 20 nm thin film of silver was selectively deposited on surface treatment with oxygen plasma, (c) selective deposition of a thin film Ag layer for SERS active sites using a shadow mask, and (f) integration of nanowell SERS structure and glass microfluidic channels.

![FIG. 1. (Color online) Fabrication procedure of the PDMS chip with Ag/PDMS nanowell structures: (a) Nanopillar fabrication on silicon as a master copy for the PDMS SERS substrate, (b) Antistiction coating on the master copy of nanowells, (c) soft lithography of nanowells on PDMS polymer, (d) surface treatment with oxygen plasma, (e) selective deposition of a thin film Ag layer for SERS active sites using a shadow mask, and (f) integration of nanowell SERS structure and glass microfluidic channels.](image)

The PDMS chip with the active SERS substrates, Ag nanowells, was stacked on and bond with a cleaned glass slide with predefined microfluidic channels as shown in [Fig. 3(a)]. The reagent solutions were introduced into the microfluidic channel from the inlet and extracted out from the outlet. The microfluidic channel has a portion in contact with the Ag/PDMS nanowell area and other portions in contact with the smooth Ag/PDMS area. Due to the semi-transparency of the Ag/PDMS substrate, the condition inside the microfluidic channel can be visualized by a conventional transmission-mode inverted microscope, which also leaves ample space for external fluidic control unit mounted on the microfluidic chip such as connectors and tubing. A 300 $\mu W$ 785 nm laser beam was focused on the Ag/PDMS surface by a 40$\times$ microscopy objective lens and the back scattered Raman signals of molecules within the detection volume are collected by the same objective lens. In the SERS experiment, the laser focal spot was intentionally illuminated on the Ag/PDMS nanowell area and smooth Ag/PDMS area respectively for comparison purposes as shown in [Figs. 3(b) and 3(c)]. The laser beam only passes the transparent optical window on the glass chip and it does not pass any thick PDMS substrate to avoid Raman scattering signals from the PDMS polymer. Both transmission-mode imaging of microfluidic channels and backscattering-mode Raman imaging of biomolecules are implemented in our microscopy system, which otherwise cannot be done if using non-transparent SERS substrate.

![FIG. 3. (Color online) (a) Schematic diagram of the integrated microfluidic chip and the biomolecular Raman imaging system. (b) Optical image of a microfluidic channel with a laser focal spot illuminated on the surface of Ag/PDMS nanowell substrate. (c) Optical image of the same microfluidic channel with a laser focal spot illuminated on the surface of smooth Ag/PDMS substrate.](image)
In order to detect steady and clear Raman signals from a very low concentration of adenosine molecules, a relatively long acquisition time, 10 s, was used. [Fig. 4(b)] shows the Raman spectra of adenosine molecules in different concentrations from 10 fM to 1 μM, the Raman spectrum after copious washing, and the Raman spectrum of 10 μM adenosine molecules on the smooth Ag/PDMS substrate. For 10 fM adenosine molecules, only the 735 cm$^{-1}$ peak is barely recognizable. With the concentration increase, more and more characteristic Raman peaks of adenosine molecules are visible besides of the increase of the peak intensities. By comparing the 735 cm$^{-1}$ peak intensity of 1 pM adenosine molecules on the nanowell area and that of 10 μM adenosine molecules on the smooth Ag/PDMS area, the Raman enhancement factor of the Ag/PDMS nanowell is at least 10$^7$ times higher than that of the smooth Ag/PDMS substrate.

Assuming the radius of the spherical detection volume, $r$, is about 2.5 μm, the diffusion rate of the small adenosine molecules (number of adenosine molecules diffusing into the detection volume per unit time) can be estimated as $\Delta N/\Delta t = 4\pi DrC$, where $D = 1 \times 10^{-6}$ cm$^2$/sec is the approximated diffusion constant of adenosine in water, and $C = 10$ fM is the molar concentration of adenosine. The diffusion rate is estimated to be ~200 molecules/sec within the detection volume. Hence within the 10-s integration time, the number of adenosine molecules excited by the focused laser beam is around 2000, which indicates that the SERS detection on the Ag/PDMS nanowell substrate has the sensitivity down to zepto mole level.

In conclusion, we have developed the robust fabrication method of silver nanowell SERS substrates by soft lithography, which allows mass production of identical SERS active sites on economical polymer substrates. The large scale monolithic integration of the Ag/PDMS nanowell in microfluidics promises label free detections of biochemical reactions in multiplexed aqueous environments.

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